Original article

Microbial Food Safety Challenges of Traditional Foods (Gueddid and Lben) of Libya

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Abstract

Gueddid (salty dry meat) and Lben (fermented butter milk) are valued Libyan traditional foods which are mostly prepared at a household level. A total of 25 samples each of Gueddid and Lben were collected from retail producers in Tripoli to evaluate their microbial safety. In Gueddid, 40% of samples had total aerobic count (TAC) 66 x 10^4 ± 73 x 10^3 CFU/g and *Enterococci* 52 x 10^4 ± 86 x 10^3 CFU/g. 20% of samples were contaminated with different *Staphylococci* 33 x 10^4 ± 58 x 10^3 CFU/g and 10% of samples contained *Staphylococcus aureus* 48 x 10^3 ± 66 x 10^2 CFU/g. 10% of samples contained Coliform 60 x 10^3 ± 80 x 10^2 CFU/g. 35% of samples contained yeast 56 x 10^3 ± 92 x 10^2 CFU/g. In Lben, all samples showed high TAC 40 x 10^6 ± 81 x 10^5 CFU/mL and all samples were contaminated with Coliform 69 x 10^5 ± 10 x 10^5 CFU/mL. 84% of samples contained *Enterococci* 13 x 10^6 ± 52 x 10^4 CFU/mL. 80% of samples had *Staphylococci* 37 x 10^5 ± 78 x 10^4 CFU/mL. 40% of samples contained *S. aureus* 46 x 10^4 ± 79 x 10^3 CFU/mL. 92% of samples contained yeast 74 x 10^5 ± 88 x 10^4 CFU/mL. Gueddid contains low microbial counts and is generally regarded as safe as it is consumed cooked. Majority of Lben samples contain high microbial load despite of their low pH. More control measures should be adopted for improving fermented food microbial safety.

Keywords: Traditional foods, Safety, Gueddid, Lben

Introduction

The fermented foods make up an important contribution to the human diet in many countries because fermentation is an inexpensive technology which preserves food, improves its nutritional value and enhances its sensory properties. The fermentation is carried out to enhance, shelf-life, texture and other attractive properties in various food of animal origin. Specially, the lactic acid fermentation inhibits growth, survival and toxin production of a number of pathogenic bacteria (Nout and Motarjemi 1997).

‘‘Gueddid’’ is one of the most appreciated traditional dry cured meats produced in Libya and regarded as a part of the artisanal staple diet in Maghreb countries. It is made of fresh lamb or goat meat strips salted, dried, and cooked in a fry pan, and then conditioned in animal fat without adding starter cultures and relying on the endogenous flora, empirically selected from the surrounding environment to keep traditional organoleptic qualities and to provide an effective form of natural preservation (Ben Belgacem *et al.* 2008; Benkerroum 2013).

Gueddid is primarily prepared from lamb meat or beef; in the subarid zones of the region, camel and goat meats are mostly used. At consumption, it is softened and desalted by immersion in water to make it tender before use as an ingredient in various dishes, such as the well-known North African couscous or legume stews (Benkerroum 2013).

Although the original purpose of transforming meat into Gueddid was its preservation to last as long as possible, given the lack of adequate storage facilities; it is now regarded as prestigious and highly prized cultural heritage food in North African countries. Therefore, local meat industries are trying to standardize its technology for an adequate transfer to industrial scale, as has been done with jerky meat (Draganski 2012), in response to consumer demands and for export to other countries.

On the other hand, a number of different types of fermented drinking products are available in many North African countries. For example, fermented buttermilk “Lben” is one of most popular drink among Libyan society (Benkerroum 2013).

The principal anti-microbial factor identified is the ability of all lactic acid bacteria (LAB) to produce organic acids and decrease the pH of foods in which they grow. Other factors such as the production of bacteriocins, hydrogen peroxide and ethanol may all play a contributory role in assuring the safety of fermented foods but their contribution is secondary (Adams and Nicolaides 1997).

Due to the lack of scientific data regarding the hygienic quality of North African traditional meat and milk products and epidemiological studies on their involvement in food outbreaks especially in Libya, this study was conducted to evaluate microbial quality of

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the locally produced Gueddid and Lben in Tripoli city to ensure whether they are safe for human consumption or not depending on the type and count of bacteria that may be present in such samples.

Materials and Methods

Sampling

A total of 50 samples of Gueddid and Lben (25 of each) were collected from different retail producers in Tripoli. The samples were sold in commercial glass jars and plastic bags and brought to the laboratory in an ice box. The samples were kept at 4 °C and analyzed within 4 hours of collection.

Determination of samples pH

The pH of the samples were measured using a JENWAY pH Meter (3505 pH Meter, Barloworld Scientific Ltd., Dunmow, UK) after calibrating using standard buffers at pH 4.0 and pH 7.0 calibration solutions (Merck Millipore).

Microbiological Analysis

Preparation of samples, decimal dilutions, culturing and enumeration techniques of bacteria were performed according to the methods described by the American Public Health Association (APHA) (Morton 2001; Swanson *et al.* 2001). Briefly, 25 g of the sample was transferred to a sterile Stomacher® bag (Stomacher 400, Seaward medicals, UK.) under aseptic conditions. The sample was then diluted to a 10-1 dilution with 225 ml peptone water (M0216, Park scientific limited, Northampton) and stomached for 2 min by using a Seward's Stomacher® 400 Circulator (Stomacher 400, Seaward medicals, UK.). Then serial dilutions were made using sterile 0.1% peptone water (Park scientific limited, Northampton). Determination of the total aerobic count (TAC) was performed using plate count agar (Difco Laboratories, Detroit, MI) which were inoculated with serial dilutions on duplicate agar plates and incubated at 37 °C for 48 h. Countable plates are those containing from 25 to 250 colonies.

Determination of Coliform count was performed using Violet Red Bile Lactose agar (VRBA, Park scientific limited, Northampton). VRBA duplicate agar plates were inoculated using an overlay method and incubated at 37 °C for 24 h. Purple-red colonies, 0.5 mm in diameter or larger, surrounded by a zone of precipitated bile acids were counted.

Enumeration of total *Staphylococci* and *Staphylococcus aureus* counts were performed using Baird-Parker agar (Park scientific limited, Northampton). The inoculums were surface plated using sterile bent glass streaking rod on duplicate agar plates. Plates were inverted and incubated at 37 °C for 48 h. Suspected *S. aureus* colonies are circular, smooth, convex, moist, 2-3 mm in diameter on un-crowded plates, gray to jet-black, frequently with light-colored (off-white) margin, surrounded by opaque zone and frequently with an outer clear zone; colonies have buttery to gummy consistency when touched with inoculating needle.

Enumeration of yeast was performed using Sabouraud's dextrose agar (CM0041, Oxoid) supplemented with chloramphenicol. The plates were inoculated using surface plating method and incubated at 25 °C for 5 days.

Enumeration of *Enterococci* was specially performed using Enterococci Selective Differential media agar (ESD) (Efthymiou *et al.* 1974). The ESD agar plates were inoculated using surface plating method and incubated at 37 °C for 24 hours.

Statistical Analysis

Analysis of variance (ANOVA) was performed by one way ANOVA with *P* value of <0.05 using Microsoft excel 2010 software.

Results and Discussion

Gueddid a typical meat product of the Maghreb countries (Morocco, Algeria, Tunisia and Libya), is obtained by a primitive technology of salting and sun-drying yielding a stable salty dry meat, which can be stored at room temperature for more than a year.

The average pH value of examined Gueddid samples was 5.90 ± 0.4. A decrease in the pH to about 5.5 was noticed at the first phase of maturation of Gueddid. Reduction of Gueddid is not efficient by itself to inhibit many pathogens; however it stimulates the growth of LAB, which, in turn, will further inhibit undesirable microorganisms through antibiosis interactions (Benkerroum 2013).

The data revealed that 40% of the examined Gueddid samples had total aerobic count (TAC) 66 x 10^4 ± 73 x 10^3 CFU/g, meanwhile the *Enterococci* count was 52 x 10^4 ± 86 x 10^3 CFU/g (Table 1 and Figure 1). In this regard, the wide occurrence of bacteriocin-producing enterococci has been reported in Tunisian Gueddid (Ben Belgacem *et al.* 2008), and the protective effect of bacteriocins in meat systems has been demonstrated (Benkerroum *et al.* 2003; Benkerroum *et al.* 2005).

In Basterma (the Egyptian style dry salty meat), the total aerobic count (TAC) and the *Lactobacillaceae* ranged between 1×10^4 and 9×10^6 CFU/g. The later range suggests that LAB are the main responsible for the evolution of the product during ripening, which represents a good indication regarding the safety of the product (El-Khateib 1997).

Gueddid, salting and drying adjunction are the main parameters used to ensure their safety and stability. While salting and/or drying reduce the water activity to levels below 0.86 where no pathogenic bacteria would grow (Jay *et al.* 2008). However, the main hazards of microbiological (pathogens and/or their toxins) associated with Gueddid are *S. aureus and Clostridium botulinum* (Bennani *et al.* 1995). Current study showed that 20% of the samples were contaminated with different *Staphylococci* 33 x 10^4 ± 58 x 10^3 CFU/g and 10% of the samples contained *Staphylococcus aureus* 48 x 10^3 ± 66 x 10^2 CFU/g (Table 1 and Fig. 1). On the other hand, only 10% of the samples contained Coliform 60 x 10^3 ± 80 x 10^2 CFU/g. Meanwhile, 35% of the samples contained yeast 56 x 10^3 ± 92 x 10^2 CFU/g.

The numbers of *Enterobacteriaceae*, yeasts and molds were less than 100 CFU/g in 50 samples of Basterma, which were also free from Salmonella (El-

Khateib 1997).

Table 1. Ranges of the microbial counts (CFU/g*) in positive Gueddid samples (Number = 25)

Groups	Positive samples %	Min	Max
TAC	40	44×10^4	88×10^4
Enterococci	40	26×10^4	78×10^4
Coliform	10	35×10^3	84×10^3
Staphylococci	20	15×10^4	50×10^4
S. aureus	10	28×10^3	68×10^3
Yeast	35	28×10^3	84×10^3

*Colony-forming unit (CFU)

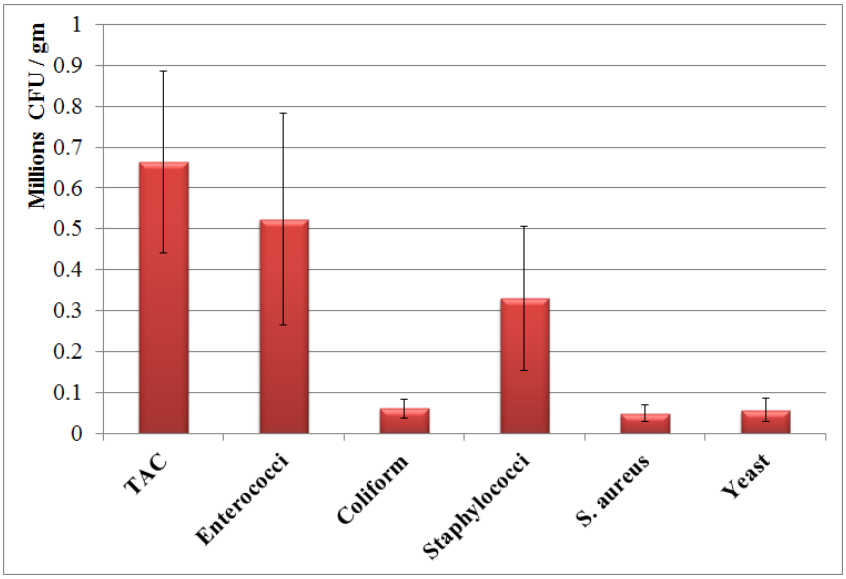

Figure 1. Average counts (CFU/g) of different microbial groups in Gueddid samples (Number = 25). Error bar represents standard deviation (SD)

In Moroccan Gueddid, the numbers of TAC, coliforms, and staphylococci showed a dramatic decrease after the maturation step to reach an undetectable level in a 1 g sample for coliforms and staphylococci, and about 40 CFU/g for TAC, this the sharp decrease in the microbial counts paralleled the decrease in water activity (aw) to a final value of 0.66 (Kalalou *et al.* 2003). Furthermore, neither Salmonella nor *Clostridium* spp. were detected in laboratory-made or commercial gueddid samples (Bennani *et al.* 1995; Bennani *et al.* 2000). However, the related salted-dried jerky meat prepared in a similar manner and having a water activity value as low as 0.3 has repeatedly been associated with a number of Salmonella and *S. aureus* outbreaks in the U.S.A. (Allen *et al.* 2007; Eidson *et al.* 2000.).

In Libyan society, Lben is prepared from raw cow's milk using naturally existing microflora. During manufacture process, a part of previously produced fermented milk is added to the raw milk at the beginning of the fermentation process. As a custom, Lben was kept in retail markets at ambient temperature until sold.

The average pH value of examined Lben samples was 4.60 ± 0.23. Nearly similar low pH range for a fermented milk product resembles Lben was measured (Gran *et al.* 2003). The low pH in the fermented milk offers a selective environment for yeast growth, but is unfavorable for most bacteria (Fleet 1990; Rohm *et al.* 1992). Spoilage becomes evident when the yeast population reaches 10^5-10^6 CFU/mL (Fleet 1990).

LAB inhibits the growth of most foodborne bacterial pathogens provided that the numbers of LAB exceed the initial level of the pathogens. However, circumstances of some pathogens are still able to grow for a limited period until the acid produced by LAB has reached inhibitory levels. Attention has focused on the ability of many bacterial pathogens such as *Salmonellae*, *Escherichia coli* and *Listeria monocytogenes* to develop acid tolerance in response to exposure to acid conditions (Rowbury 1995).

All of the examined Lben samples contain high TAC $40 \times 10^6 \pm 81 \times 10^5$ CFU/mL despite of the low pH of the product (Table 2 and Figure 2). Therefore, Lben produced by traditional methods may have microorganisms other than lactic acid bacteria playing a significant role in the fermentation process.

Coliforms were found in all examined Lben samples (Table 2). Whereas, the average of Coliforms counts were 69 x 10^5 ± 10 x 10^5 CFU/mL (Figure 2). Presence of Coliforms in food is an indicator for fecal contamination (Naas *et al.* 2007). The high level of coliform contamination of milk might be due to initial contamination originating from the udder surface, wash water, or milking utensils. Further contacts with other possible sources of contamination are added to the sources of contamination in case of fermented milk before, during and after manufacture. This may explain the high incidence and high mean count for Coliforms. Nevertheless, the presence and growth of Coliform may lead to a public health hazard (Garbaj *et al.* 2007).

Enterococci were found in 84% of the examined Lben samples (Table 2). Whereas, the *Enterococci* mean count was 13 x 10^6 ± 52 x 10^4 CFU/mL (Fig. 2). Presence of *Enterococci* is indicative for fecal contamination. Consequently it also indicates unsatisfactory production and handling (Moawad and El- Neary 1996). *Enterococci* are also implicated in food poisoning and can cause serious illness in human (Hoffmann and Moellering 1987).

Total *Staphylococci* and *S. aureus* were found in 80% and 40% of the examined Lben samples respectively (Table 2). The presumptive Staphylococci mean count was 37 x 10^5 ± 78 x 10^4 CFU/mL. Furthermore the *S. aureus* mean count was 46 x 10^4 ± 79 x 10^3 CFU/mL (Figure 2).

The determined counts of total *Staphylococci* and *S. aureus* are unacceptable in comparison with the permissible limits suggested by the Libyan standard specifications.

Table 2. Ranges of the microbial counts (CFU/g*) in positive Lben samples (Number = 25)

Groups	Positive samples %	Min.	Max.
TAC	100	16 x 10^6	65 x 10^6
Enterococci	84	11 x 10^6	15 x 10^6
Coliform	100	38 x 10^5	10 x 10^6
Staphylococci	80	14 x 10^5	61 x 10^5
S. aureus	40	22 x 10^4	70 x 10^4
Yeast	92	48 x 10^5	10 x 10^6

*Colony-forming unit (CFU)

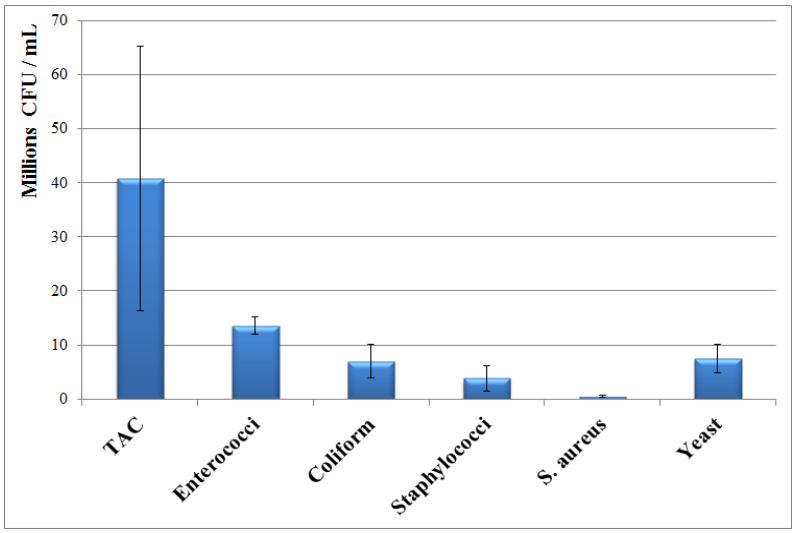

Figure 2. Average counts (CFU/mL) of different microbial groups in Lben samples (Number = 25). Error bar represents standard deviation (SD)

The high *Staphylococci* counts in Lben may be due to the unhygienic measures during handling, processing or transportation of fermented milk. Presence of *S. aureus* in higher number may cause food poisoning due to production of enterotoxins (Freed *et al.* 1982).

Yeast was found in 92% of the examined Lben samples (Table 2). Total yeast mean count was 74 x 10^5 ± 88 x 10^4 CFU/mL (Figure 2).

Such high numbers of yeasts suggests that the yeasts are able to multiply in the milk and may result in spoilage or, conversely, in enhancement of the flavor of the fermented milk. As there was no definite time for fermentation step during manufacture of Lben samples studied in current work, thus greatly variable counts were obtained in this study. However, as the longer the fermentation time, the higher the yeast count is detected in fermented milk products (Gadaga *et al.* 2000). There

is increasing interest in the role of yeasts in dairy fermentation and especially their potential use as starter cultures (Fleet 1990; Jakobsen and Narvhus 1996).

For controlling of such contaminations in Gueddid and Lben, it is important to focus the attention upon the quality of the raw meat and milk before processing to ensure the quality of the end product. Also, the unsatisfactory conditions of processing, handing and distribution of traditionally made Gueddid and Lben, in addition to the lack of efficient veterinary supervision upon foods originated from animal may explain the obtained result in this work.

Health education of food handlers, consumer perception of fermented meat and milk products, characterization and optimization of fermentation processes and development of appropriate starters could reduce the likelihood of higher microbial count and possibility of associated outbreaks.

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References

Adams MR and Nicolaides L (1997). Review of the sensitivity of different foodborne pathogens to fermentation. *Food Control* 8**:** 227-239.

Allen K, Cornforth D, Whittier D, Vasavada M and Nummer B (2007). Evaluation of high humidity and wet marinade methods for pasteurization of jerky. *J Food Sci*. 72**:** C351-355.

Ben Belgacem Z, Ferchichi M, Prevost H, Dousset X and Manai M (2008). Screening for anti-listerial bacteriocin-producing lactic acid bacteria from "Gueddid" a traditionally Tunisian fermented meat. *Meat Sci*. 78**:** 513-521.

Benkerroum N (2013). Traditional Fermented Foods of North African Countries: Technology and Food Safety Challenges With Regard to Microbiological Risks. *Compr Rev Food Sci Food* Safety 12**:** 54-89.

Benkerroum N, Daoudi A, Hamraoui T, Ghalfi H, Thiry C, Duroy M, Evrart P, Roblain D and Thonart P (2005). Lyophilized preparations of bacteriocinogenic Lactobacillus curvatus and Lactococcus lactis subsp. lactis as potential protective adjuncts to control Listeria monocytogenes in dry-fermented sausages. *J Appl Microbiol*. 98**:** 56-63.

Benkerroum N, Daoudi A and Kamal M (2003). Behaviour of Listeria monocytogenes in raw sausages (merguez) in presence of a bacteriocin-producing lactococcal strain as a protective culture. *Meat Sci*. 63**:** 479-484.

Bennani L, Faid M and Bouseta A (2000). Experimental manufacturing of kaddid, a salted dried meat product: control of the microorganisms. *Eur Food Res Technol*. 211**:** 153-157.

Bennani L, Zenati Y, Faid M and Ettayebi M (1995). Physico-chemical and microbiological characteristics of a dried salted meat product (Kaddid) in Morocco. *Z Lebensm Unters Forsch*. 201**:** 528-532.

Draganski A (2012) Dried meat snack and process of preparation thereof. Google Patents.

Efthymiou CJ, Baccash P, Labombardi VJ and Epstein DS (1974). Improved isolation and differentiation of enterococci in cheese. *Appl Microbiol*. 28**:** 417-422.

Eidson M, Sewell CM, Graves G and Olson R (2000.). Beef jerky gastroenteritis outbreaks. *J Environ Health*. 62**:** 9-13.

El-Khateib T (1997). Microbiological status of Egyptian salted meat (Basterma) and fresh sausage. *J Food Safety*. 17**:** 141-150.

Fleet GH (1990). Yeasts in dairy products. *J Appl Bacteriol*. 68**:** 199-211.

Freed RC, Evenson ML, Reiser RF and Bergdoll MS (1982). Enzyme-linked immunosorbent assay for detection of staphylococcal enterotoxins in foods. *Appl Environ Microbiol*. 44**:** 1349-1355.

Gadaga TH, Mutukumira AN and Narvhus JA (2000). Enumeration and identification of yeasts isolated from Zimbabwean traditional fermented milk. *Int Dairy J*. 10**:** 459-466.

Garbaj AM, Naas HT, Gammoudi FT and Moawad AA (2007). Bacteriological quality of Mozzarella cheese sold in Tripoli Governorate. *Benha Vet Med J*. 17**:** 99 – 104.

Gran HM, Gadaga HT and Narvhus JA (2003). Utilization of various starter cultures in the production of Amasi, a Zimbabwean naturally fermented raw milk product. *Int J Food Microbiol*. 88**:** 19-28.

Hoffmann SA and Moellering JRC (1987). The Enterococcus: "Putting the Bug in Our Ears". *Ann Intern Med*. 106**:** 757-761.

Jakobsen M and Narvhus J (1996). Yeasts and their possible beneficial and negative effects on the quality of dairy products. *Int Dairy J*. 6**:** 755-768.

Jay JM, Loessner MJ and Golden DA (2008) *Modern Food Microbiology*, (7th Ed)Springer.

Kalalou I, Faid M and Ahami TA (2003). Control of hazardous microorganisms in “kaddid” from camel meat. *J Camel Pract Res*. 10**:** 163–167.

Moawad AA and El- Neary NA (1996). Microbial studies on creamy "Kishda" cheese sold in Egyptian markets. *Benha Vet Med J*. 2**:** 111 - 115.

Morton RD (2001) Aerobic Plate Count. In *Compendium of Methods for The Microbiological Examination of Foods*, F.P. Downes, K. Ito (eds.) 4th edn., American Public Health Association, pp. 63-67.

Naas HT, Gammoudi FT, Garbaj AM, Azwai SM and Moawad AA (2007). Comparison between two different conventional methods for coliform count in raw milk and locally made soft cheese in Tripoli-Libya. *Benha Vet Med J*. 18**:** 129 - 138.

Nout MJR and Motarjemi Y (1997). Assessment of fermentation as a household technology for improving food safety: a joint FAO/WHO workshop. *Food Control* 8**:** 221-226.

Rohm H, Eliskases-Lechner F and Bräuer M (1992). Diversity of yeasts in selected dairy products. *J Appl Microbiol*. 72**:** 370-376.

Rowbury RJ (1995). An assessment of environmental factors influencing acid tolerance and sensitivity in Escherichia coli, Salmonella spp. and other enterobacteria. *Lett Appl Microbiol*. 20**:** 333-337.

Swanson KMJ, Petran RL and Hanlin JH (2001) Culture Methods for Enumeration of Microorganisms. In *Compendium of Methods for The Microbiological Examination of Foods*, F.P. Downes, K. Ito (eds.). American Public Health Association, pp. 53-62.